IMPACT OF SEED HARDENING CHEMICALS TO INDUCE DROUGHT TOLERANCE IN CHICKPEA (*Cicer arietinum* L.)

Abstract :

A field experiment was conducted at the Main Agriculture Research Station, University of Agricultural Sciences, Dharwad during rabi 2021 to evaluate the impact of seed hardening with Aminolevulinic acid (ALA) on Physiological, Biochemical, and yield parameters of chickpea (var. JG-11). The experiment, laid out in a randomized block design with three replications, included seed soaking treatments with ALA at concentrations of 10, 20, 30, 40, and 50 ppm, along with 2% $CaCl_2$. Results indicated that ALA at 30 ppm significantly improved plant height, number of primary branches, total dry matter, and SPAD value compared to the control. Additionally,By showing high relative water content, Proline content, Chlorophyll content and chlorophyll stability index. Seed hardening with ALA (30 ppm) and $CaCl_2$ (2%) also resulted in increased yield components and yield, with the highest economic returns observed in the 2% $CaCl_2$ treatment. Overall, the study demonstrated that seed hardening with ALA (30 ppm) and $CaCl_2$ (2%) effectively enhanced drought tolerance and improved chickpea productivity.

Introduction

Chickpea (*Cicer arietinum* L.), a self-pollinated legume with a chromosome number of 2n=16, is a vital Rabi season pulse crop in India and a significant source of protein in vegetarian diets. It is a small, branched herbaceous plant that rarely exceeds 60 cm in height. Pulses, including chickpea, play a crucial role in Indian agriculture, with India being the world's largest pulse-producing country. In 2014-15, India was projected to produce 264.38 million tons of food grains, with chickpea being one of the most prominent crops. Globally, chickpea is the second largest grown pulse crop, with 84.5% of production coming from Asia, particularly from Southern Asia. During 2013, the global production of chickpea reached 13.1 million metric tons, with significant contributions from countries in Asia, Oceania, Africa, the Americas, and Europe.

Drought is a major abiotic stress that significantly affects chickpea productivity. Water stress, particularly during anthesis to maturity, hampers various morphological and physiological processes in plants, leading to reduced yields. One effective strategy to mitigate drought stress is pre-sowing seed hardening, a technique involving repeated soaking of seeds in solutions

containing organic or inorganic solutes, followed by redrying. This treatment enhances seed vigor by facilitating pre germinative metabolic activity while preventing radical emergence. Seed hardening has been shown to improve seed performance under stress conditions by protecting the plasma membrane structure, which is crucial for maintaining cellular integrity during drought.

Several studies have highlighted the positive effects of seed hardening on chickpea, including enhanced drought tolerance, increased seed germination, and improved seedling emergence. The technique improves seed vigor, protects against membrane injury under stress, and enhances the development of a stronger root system. Seed priming and hardening are common practices in India to enhance seed performance, especially under challenging environmental conditions.

Materials and methodology

A field experiment was conducted during rabi 2021-22 to study the impact of seed hardening chemicals to induce drought tolerance in chickpea with water, $CaCl_2$ and Aminolevulinic acid on Physiological, biochemical, yield and yield components in chickpea (*Cicer arietinum* L.) at College of Agriculture Farm, University of Agricultural Sciences, Dharwad.The experimental site consisted of medium black clay loam soil.The experiment consisted of eight seed hardening treatments viz., water, $CaCl_2$(2%) and Aminolevulinic acid with a control on genotype JG-11.

Relative water content

The relative content was estimated by the method of Barrs was Weatherly (1962). Ten leaf discs were collected randomly in each treatment and weighed accurately up to third decimal on a single pan analytical balance. This was considered as fresh weight. The weighed leaf discs were allowed to float on distilled water in a Petri dish and allowed to absorb water for four hours. After four hours, the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to as turgid weight. After drying in hot air oven at 72^0 C for 48 hours, the dry weight was recorded and RWC was calculated by using the following formula,

$$RWC\ (\%) = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \times 100$$

Relative chlorophyll content (SPAD)

The SPAD (Soil Plant Analysis Development) chlorophyll meter is a simple, rapid, and non-destructive method for evaluation of chlorophyll contents in leaves and can be used in the field and laboratory that gives the relative chlorophyll content and greenness in leaves in terms of SCMR (SPAD Chlorophyll meter reading) values. The SCMR values were recorded in the standard leaf (third fully opened leaf from shoot tip on main stem) of all plants and mean was recorded. Such SCMR value was recorded in different stages viz., 30,45, 60, DAS.

Chlorophyll stability index (CSI)

Green plants pigments are thermo-sensitive and degradation occurs when they are subjected to higher temperature. This method is based on pigment changes induced by heating. Chlorophyll stability is the function of temperature and this property of chlorophyll stability was found to have good correlation with drought resistance. Representative leaf sample was placed in two clean tubes with 50 ml of distilled water. One tube was then subjected to heat on water bath at $56^{o}C \pm 1^{o}C$ for exactly 30 minutes. The chlorophyll in both the samples was extracted by placing the sample in 7 ml of DMSO at $65^{o}C$ for 30 minutes. The supernatant was decanted and the tissue was discarded, then volume was made to 10 ml by DMSO. Finally, the absorbance of the extract was read at 645, and 663 nm using DMSO as blank (Hiscox and Isrealstam , 1979).

$$\text{Total chlorophyll (mg g-1 fr.wt.)} = 20.2 \times (A_{645}) - 8.02 \times (A_{663}) \times \frac{V}{1000 \times w \times a}$$

where,

A645 = Absorbance of the extract at 645 nm

A663 = Absorbance of the extract at 663 nm

a = Path length of cuvette (1 cm)

V = final volume of the chlorophyll extract (10 ml)

W = Fresh weight of the sample (0.10 g)

$$CSI\ (\%) = \frac{C_S}{C_C} \times 100$$

where,

CSI = chlorophyll stability index

Cs = chlorophyll content of stressed plant

Cc = chlorophyll content of control plant

a = Path length of cuvette (1 cm)

Estimation of proline

Proline content was estimated by following the method of Bates et al. (1973). A known weight (0.5 g) of fresh leaf sample was macerated in a mortar using 10 ml of 3 per cent sulphosalicylic acid. The extract was filtered and 2.0 ml of the filtrate was used for proline estimation. To this 2.0 ml of filtrate, 2.0 ml of acid ninhydrin reagent (2.5 g of ninhydrin dissolved in 40 ml of 6.0 M orthophosphoric acid and 60 ml of glacial acetic acid), 2.0 ml of glacial acetic acid were added and placed in boiling water bath for one hour. Following this, test tubes containing the samples were transferred to an ice bath for cooling. The contents of each test tube were transferred to a separatory funnel and 6.0 ml of toulene was added, shaken thoroughly and allowed for few minutes for separation of two layers. The lower layer was discarded and the upper toulene layer containing the colour complex was taken into a test tube. The optical density was read at 520 nm using spectrophotometer (Elico, UV-vis spectrophotometer) and the proline content was calculated as follows.

$$\text{Proline content (mg g}^{-1}\text{ fresh weight)} = \frac{36.2311 \times OD \times V \times d}{2 \times f}$$

where,

OD = Optical density at 520 nm

V = Volume of aliquot made (ml)

D = Fresh weight / dry weight ratio

f = Fresh weight taken for proline estimation (mg)

Result and Discussion

Relative water content (RWC) (%)

The results concerned to relative water content varied significantly among the treatments. Relative water content (RWC) increased up to 45 DAS and decreased thereafter. At 30 DAS, ALA at 30 ppm (75.8%) showed the highest RWC, followed by CaCl2 at 2% (74.3%) and ALA at 20 ppm (72.1%), with the control at 64.9%. A similar trend was observed at 45 DAS.

By 60 DAS, RWC declined overall, with ALA at 30 ppm still showing the highest RWC (71.2%), while the control had the lowest (53.4%).The influence of seed hardening treatments was investigated in this direction. Relative water content (RWC) is a measure of the amount of water present in leaf tissue in relation to turgid condition, and treatments with higher RWC under drought conditions are preferable to maintain higherwater balance.In the current study, seed hardening with Aminolevulinic acid (30ppm) recorded significantly higher RWC values followed by $CaCl_2$ (2%) as compared to control. These findings are consistent with Manjunath and Dhanoji (2011)in chickpea.

SPAD values

At 30 DAS, the highest SPAD value was recorded with ALA at 30 ppm (39.66), followed by CaCl2 at 2% (37.45) and ALA at 20 ppm (35.55), with the control at 29.36. At 45 DAS, ALA at 30 ppm (47.20) had the highest SPAD value, followed by CaCl2 at 2% (43.02). At 60 DAS, ALA at 30 ppm still recorded the highest SPAD value (48.94), with the control at 36.91.SPAD values increased up to 60 DAS, with the highest values recorded in seed hardening with Aminolevulinic acid (30 ppm), followed by CaCl2 (2%). Hotta et al. (2004) noted that ALA is a precursor in chlorophyll biosynthesis. Similar results were reported by Kumeera*et al*. (2018) in chickpea and Suliman *et al*. (2021) in wheat. These treatments positively influenced SPAD values, improving chlorophyll content.

Chlorophyll stability index (CSI) (%)

The highest Chlorophyll Stability Index (CSI) was recorded with Aminolevulinic acid (ALA) at 30 ppm (73.8%), followed by CaCl2 at 2% (71.8%) and ALA at 20 ppm (70.2%), with the control at 44.3%. At 45 DAS, ALA at 30 ppm recorded the highest CSI (75.3%), followed by CaCl2 at 2% (73.1%). At 60 DAS, ALA at 30 ppm again showed the highest CSI (77.1%), followed by CaCl2 at 2% (75.8%), with the control at 48.7%.The chlorophyll stability index (CSI) was raised from 30 to 60 DAS. Seed hardening with Aminolevulinic acid (30ppm) recorded significantly higher CSI than control. Because chlorophyll stability varies with temperature, the destruction of chlorophyll pigment changes in this case due to terminal stress. This property of chlorophyll stability was reported to correlatewellwithdroughtresistance.HighCSIwasassociatedwithgreaterdroughttolerance Kumeera*et al.* (2018).

Proline content (µg g^{-1} fresh weight)

At 30 DAS, the highest proline content was recorded with Aminolevulinic acid (ALA) at 30 ppm (128 µg g^{-1} fw), followed by CaCl2 at 2% (123 µg g^{-1} fw) and ALA at 20 ppm (115 µg g^{-1} fw), with the control at 93 µg g^{-1} fw. At 45 DAS, the maximum proline content was observed with ALA at 30 ppm (173 µg g^{-1} fw), followed by CaCl2 at 2% (169 µg g^{-1} fw). At 60 DAS, ALA at 30 ppm recorded the highest proline content (220 µg g^{-1} fw), followed by CaCl2 at 2% (214 µg g^{-1} fw), with the control at 164 µg g^{-1} fw.Desiccationhasbeenshowntoincreasetheamountoffreeprolineintheleavesofmany plant species. Suliman *et al*. (2021) hypothesised that proline accumulation could contribute to osmotic adjustment. According to Zhang *et al*. (2012), proline plays an important role in the storage of carbon and nitrogen, the detoxification of NH_3, the preservation of protein hydration in dehydrated tissues, and the survival of cellularfunctions.Seed hardening with Aminolevulinic acid (30 ppm) and CaCl2 (2%) increased proline content significantly compared to the control. These findings align with Manjunath and Dhanoji (2011) in chickpea and Suliman et al. (2021) in wheat.

100 seed weight(g)

Among the treatments Aminolevulinic acid @ 30ppm recorded significantly highest 100 seed weight (31.41 g) followed by $CaCl_2$ @ 2 per cent (30.12 g) and Aminolevulinic acid @ 20 ppm (29.21 g) whereas, the lowest 100 seed weight was recorded in control (23.49 g).study found that seed hardening chemicals increased seed yield and100seedweight

Seed yield (q ha^{-1})

The seed yield differed significantly between the treatments. Seed hardening with Aminolevulinic acid @ 30ppm recorded significantly higher seed yield (26.1 q ha^{-1})followed by 2% $CaCl_2$ (25.2 q ha^{-1}) and Aminolevulinic acid @ 20 ppm (24.8 q ha^{-1}) while, significantly lowest seed yield was recorded in control (19.14 q ha^{-1}). Seed hardening treatments improved seed yield by enhancing water absorption, photosynthesis, and tissue hydration, allowing better resistance to soil moisture stress (Prajapati et al., 2017). Sher et al. (2021) found that ALA (75

mg L^{-1}) increased sunflower yield. Prajapati et al. (2017) also reported higher pod numbers and pod yield in blackgram with $CaCl_2$ (2%). These results suggest seed hardening improves overall plant performance and yield.

Table 1:Influence of seed hardening chemicals on SPAD value at different stages in chickpe

Treatment	SPAD		
	30DAS	**45DAS**	**60DAS**
T**1**: Seed soaking with (5-ALA) @ 10 ppm	34.7	37.34	40.91
T**2**: Seed soaking with (5-ALA) @ 20 ppm	35.55	38.31	42.06
T**3**: Seed soaking with (5-ALA) @ 30 ppm	39.66	47.20	48.94
T**4**: Seed soaking with (5-ALA) @ 40 ppm	33.26	36.6	39.62
T**5**: Seed soaking with (5-ALA) @ 50 ppm	31.49	35.91	38.6
T**6**: Seed soaking with $CaCl_2$ @ 2 %	37.45	43.02	46.21
T**7:** Seed soaking with water	30.85	35.6	38.02
T**8**: Control	29.36	34.77	36.91
Mean	**34.04**	**38.61**	**41.41**
S.Em±	1.04	1.20	1.28
CD (5%)	3.17	3.64	3.88

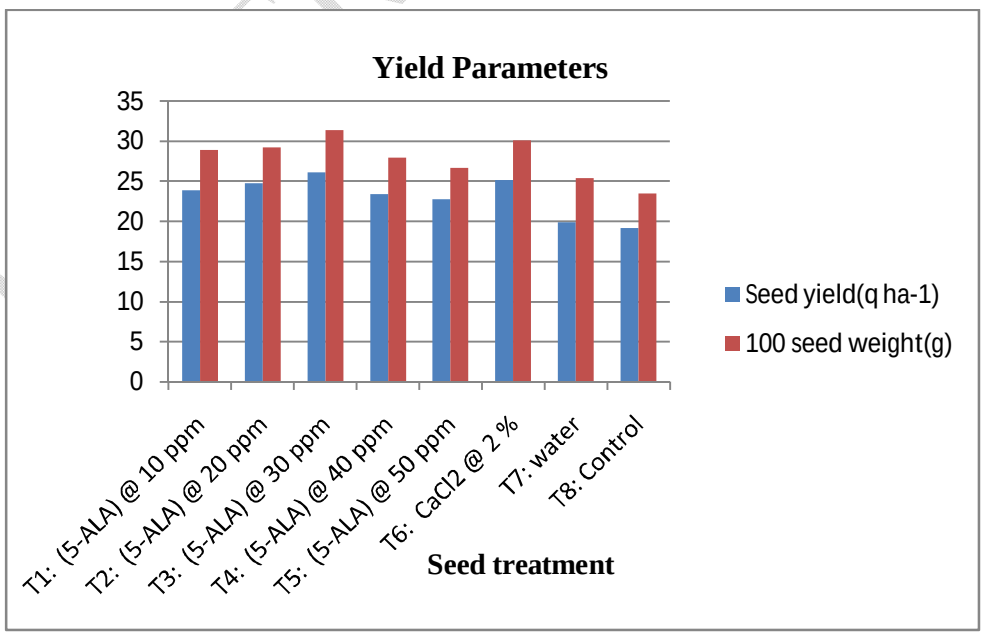
Table 2: Influence of seed hardening chemicals on chlorophyll stability index (%) at different stages in chickpea

Treatment	CSI		
	30DAS	**45DAS**	**60DAS**
T**1**: Seed soaking with (5-ALA) @ 10 ppm	68.	70.4	73.1
T**2**: Seed soaking with (5-ALA) @ 20 ppm	70.2	71.8	74.3
T**3**: Seed soaking with (5-ALA) @ 30 ppm	73.8	75.3	77.1
T**4**: Seed soaking with (5-ALA) @ 40 ppm	66.5	68.6	71.5
T**5**: Seed soaking with (5-ALA) @ 50 ppm	64.1	67.4	70.2
T**6**: Seed soaking with $CaCl_2$ @ 2 %	71.8	73.1	75.8
T**7:** Seed soaking with water	46.5	49.7	52.2
T**8**: Control	44.3	46.2	48.7
Mean	**63.26**	**65.31**	**67.86**
S.Em ±	1.95	2.01	2.09
CD (5%)	5.93	6.10	6.33

Table 3: Influence of seed hardening chemicals on Proline content (mg g^{-1} fresh weight) at different stages in chickpea

Treatment	Proline		
	30DAS	**45DAS**	**60DAS**
T**1**: Seed soaking with (5-ALA) @ 10 ppm	112	155	201
T**2**: Seed soaking with (5-ALA) @ 20 ppm	115	157	204
T**3**: Seed soaking with (5-ALA) @ 30 ppm	128	173	220
T**4**: Seed soaking with (5-ALA) @ 40 ppm	109	151	196
T**5**: Seed soaking with (5-ALA) @ 50 ppm	104	149	189
T**6**: Seed soaking with $CaCl_2$@ 2 %	123	169	214
T**7:** Seed soaking with water	98	144	168
T**8**: Control	93	139	164
Mean	**110.25**	**154.62**	**194.5**
S.Em ±	3.3	4.7	5.9
CD (5%)	10.2	14.3	18.0

Fig 1:Influence of seed hardening chemicals on yield and yield components in chickpea



Conclusion

Seed hardening is a simple, easy, and inexpensive way to improve seed performanceand agricultural productivity, particularly in resource-poor farmers' drylands and marginallands. In conclusion, seed hardening enhances drought tolerance by altering key physiological and biochemical factors. Treatments like Aminolevulinic acid (ALA) and $CaCl_2$ improve water absorption, photosynthetic activity, and tissue hydration, helping plants better withstand soil moisture stress. Additionally, they increase proline content and chlorophyll stability, contributing to improved plant performance and higher yields under drought conditions. These findings suggest that seed hardening can be a valuable strategy for improving drought resilience in crops.Accordingtotheresultsofvariousseedhardeningtreatments,itisconcludedthatseedhardeningwith30 ppmALAand2%$CaCl_2$is moreeffectiveinincreasing chickpeayield.

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